

Involvement of Neuropeptides in the Allergic Nasal Obstruction in Guinea Pigs

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ABSTRACT—The purposes of the present study were i) to determine whether neuropeptides induce the nasal obstruction in guinea pigs, and ii) to examine the possible involvement of neuropeptides in allergic nasal obstruction. The decrease in nasal cavity volume was determined by acoustic rhinometry as an index of nasal obstruction. In non-sensitized guinea pigs, substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) caused the nasal obstruction 10 to 30 min after their intranasal application. LY303870 (1 mg/kg), a tachykinin NK₁-receptor antagonist; SR48968 (1 mg/kg), a tachykinin NK₂-receptor antagonist; and CGRP(8–37) (50 nmol/kg), a CGRP1-receptor antagonist, administered intravenously before the intranasal application of the neuropeptides, inhibited the responses induced by SP, NKA and CGRP, respectively. In the guinea pigs sensitized with dinitrophenyl-coupled *Ascaris suum* allergenic extract, the intranasal antigen challenge caused nasal obstruction. The response was biphasic and consisted of the early phase response (EPR) and the late phase response (LPR), which developed 30 min and 6 h, respectively, after the antigen challenge. Intravenous administration of LY303870 (1 mg/kg) before the antigen challenge inhibited the EPR, while those of SR48968 (1 mg/kg) and CGRP(8–37) (50 nmol/kg) inhibited the LPR. The present results suggest that neuropeptides are involved in the allergic nasal obstruction.

Keywords: Nasal obstruction, Substance P, Neurokinin A, Calcitonin gene-related peptide

Nasal obstruction is one of the major symptoms of allergic rhinitis. The previous studies in allergic rhinitis patients demonstrated that classical histamine H₁-receptor antagonists such as chlorpheniramine, hydroxyzine and clemastine were not effective in the treatment of nasal obstruction (1, 2), suggesting an involvement of mediators other than histamine in the pathogenesis of the nasal obstruction. The involvement of peptide leukotrienes (p-LTs) and thromboxane A₂ has been suggested as pranlukast (3), a p-LTs receptor antagonist, and ramatroban (4), a thromboxane A₂-receptor antagonist, showed clinical efficacy in the treatment of nasal obstruction.

There are some results suggesting the involvement of neuropeptides in the pathogenesis of nasal obstruction, although the effectiveness of an antagonist against neuropeptide receptor has not been elucidated in humans. Nerve fibers containing neuropeptides such as substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) have been found in the human nasal mucosa (5, 6).

Their receptors, that is, tachykinin NK₁, tachykinin NK₂ and CGRP1 receptors, respectively, have also been shown to exist in human nasal mucosa (5, 6). Moreover, the intranasal administration of SP, NKA or CGRP has been reported to cause nasal obstruction in humans (7–9).

The purposes of the present study were i) to determine the effects of SP, NKA and CGRP on the nasal cavity volume in guinea pigs and ii) to examine the possible involvement of the neuropeptides in the nasal obstruction in a guinea pig model of allergic rhinitis. First, since the nasal obstruction after intranasal application of neuropeptides in guinea pigs has not been reported, we investigated whether the 3 neuropeptides cause nasal obstruction in non-sensitized guinea pigs; and if so, which receptor is involved in each response by using the corresponding neuropeptide antagonists. Second, we examined the effects of neuropeptide antagonists on the nasal obstruction following the intranasal antigen challenge in actively sensitized guinea pigs. In this study, we employed LY303870 (10), a tachykinin NK₁-receptor antagonist, SR48968 (11), a tachykinin NK₂-receptor antagonist and CGRP(8–37) (12), a CGRP1-receptor antagonist.

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MATERIALS AND METHODS

Animals

Male Hartley guinea pigs, 4-weeks-old, were purchased from Japan SLC (Shizuoka) and were used for the experiments after being kept in the holding room in our laboratories for several days. The animals were housed at a room temperature of 19–25°C and a relative humidity of 30–70% with a 12-h light-dark cycle (lights on at 7:00). Food and water were freely available. The present experiments were approved by the Animal Ethical Committee of Kyowa Hakko Kogyo Co., Ltd. (Shizuoka).

Drugs

LY303870 ((R)-1-[N-(2-methoxybenzyl)acetyl-amino]-3-(1*H*-indol-3-yl)-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]-propane) and SR48968 ((S)-*N*-methyl-*N*[(4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) were synthesized at the Pharmaceutical Research Institute of Kyowa Hakko Kogyo Co., Ltd. (Shizuoka). SP, NKA, CGRP, CGRP(8–37) and bovine serum albumin (BSA) were purchased from Sigma Chemicals (St. Louis, MO, USA). LY303870 and CGRP(8–37) were dissolved in saline, and SR48968 was suspended in saline containing 0.5 vol% of Tween 80. SP, NKA and CGRP were dissolved in 0.1 w/v% BSA in saline. Other drugs used in this study were *Ascaris suum* allergenic extract (Funakoshi, Tokyo); 2,4-dinitrobenzenesulfonic acid sodium salt, and carbamic acid ethylester (urethane) (Tokyo Kasei, Tokyo); aluminium sulfate and 20 w/v% sodium hydroxide (Wako Pure Chemical, Osaka).

Evaluation of nasal obstruction in guinea pigs

Nasal obstruction was evaluated by observing the decrease in nasal cavity volume, which was measured by acoustic rhinometry in guinea pigs under anesthesia with urethane (1.2 mg/kg, i.p.). We used the acoustic rhinometer for guinea pigs (GJ Elektronik, Skanderborg, Denmark). The details of the acoustic reflection technique were reported elsewhere (13). In rhinometry, a sound pulse created by a spark in a sound tube connected with the nasal cavity reflects from the nasal cavity, and the acoustic reflections are measured as a function of the distance from the nostril. From this reflection curve, the nasal cavity volume between the nostril and 2 cm into the nasal cavity was calculated with a computer for each measurement. For each animal, the mean value of 3 measurements for each nostril was regarded as the volume. The nasal obstruction of each animal was evaluated by the sum of the decreases in volume of the left and right nasal cavities. The nasal obstruction was expressed as the percentage change from the basal nasal cavity volume.

Effects of neuropeptides on the nasal cavity volume in non-sensitized guinea pigs

Non-sensitized guinea pigs were anesthetized with urethane and 20 μ l of SP, NKA or CGRP solution was applied into bilateral nostrils. The nasal cavity volume was measured before and until 30 min after the application, since the nasal obstruction following SP application was reported to last for less than 20 min in humans (9).

Antigen-antibody reaction at the nose

The method reported by Ishida et al. (14) was used with some modifications. *Ascaris suum* allergenic extract coupled with dinitrophenyl (DNP-*Ascaris*) by the method of Eisen et al. (15) was used as an antigen. Guinea pigs were actively sensitized by intraperitoneal injections of DNP-*Ascaris* (3.12 μ g protein) with alum 4 times at 2-week intervals. The sensitization was boosted by nebulization of DNP-*Ascaris* (15.6 μ g protein/ml, for 3 min) beginning 2 weeks after the fourth intraperitoneal injection, and this procedure was repeated every day for 5 days. Animals were used at least 10 days after the final nebulization. The IgG and IgE antibody titers of sera obtained from the sensitized animals were estimated by the 4-h and the 8-day homologous passive cutaneous anaphylaxis (PCA), respectively, in guinea pigs. The IgG antibody titers were between 1:50 to 1:1600, while the IgE antibody titers ranged from 1:50 to 1:200. The 8-day PCA titers of the sera heated at 56°C for 2 h were lower than those without the treatment, and thus the antibodies estimated by the 8-day PCA were confirmed to be the IgE antibodies. Antigen-antibody reaction at the nose was produced by the administration of 20 μ l of DNP-*Ascaris* solution (1.8 mg protein/ml) into bilateral nostrils.

Effects of antagonists against neuropeptides on nasal obstruction

LY303870, SR48968 and CGRP(8–37) were used as antagonists against NK₁-, NK₂- and CGRP1-receptors. Their selectivity has been confirmed as described below. LY303870 blocked the NK₁-receptor-mediated response, that is, the SP-induced contractile response of rabbit cava vein with a pA₂ of 9.4, while this drug was approximately 50,000-fold less effective against the NK₂- or NK₃-receptor-mediated response (10). SR48968 inhibited the NK₂-receptor-mediated response, that is, the [β Ala⁸]-NKA(4–10)-induced contraction of rabbit pulmonary artery with a pA₂ of 10.3, while this drug was inactive up to a concentration of 100 nmol/l against the NK₁- or NK₃-receptor-mediated response (11). CGRP(8–37) inhibited the CGRP1-receptor-mediated response; that is, the human CGRP α -induced increase in contractile force of guinea pig left atrium had a pA₂ of 7.66, while this drug was tenfold less active against the CGRP2 receptor-mediated response (16).

In non-sensitized guinea pigs, LY303870 or SR48968 was intravenously administered 30 min before, and CGRP(8–37) was intravenously given 2, 10 or 30 min before the intranasal challenge with the neuropeptides, since CGRP(8–37) may exhibit short duration of action in vivo (12). The nasal cavity volume was measured before and until 30 min after the challenge. In sensitized guinea pigs, LY303870 or SR48968 was intravenously administered 30 min before, and CGRP(8–37) was intravenously given 2 min before the intranasal antigen challenge. The nasal cavity volume was measured before and until 6 h after the challenge, since our previous study demonstrated that the decreases in the nasal cavity volume developed till 6 h after the antigen challenge in sensitized guinea pigs (17).

We used these antagonists at doses sufficient to antagonize each receptor: LY303870 at 1 mg/kg, SR48968 at 1 mg/kg and CGRP(8–37) at 50 nmol/kg. LY303870 given intravenously at 1 mg/kg is reported to produce nearly complete inhibition of [Sar⁹,Met(O₂)¹¹]-SP-induced bronchoconstriction in guinea pigs (10). Intravenous administration of SR48968 at 0.6 mg/kg completely inhibited [β Ala⁸]-NKA(4–10)-induced bronchoconstriction in guinea pigs (18). CGRP(8–37) given intravenously at 50 nmol/kg abolished the enhancement by CGRP of dry gas hyperpnea challenge-induced change in lung resistance (12).

Statistical analyses

Data are shown as means $\pm$ S.E.M. The Student's *t*-test or the Aspin-Welch test was used for the analysis of the difference between the control and the drug group at each time, and the Steel's test was used when the drug group was plural. A value of *P* less than 5% was considered to be significant.

RESULTS

Effects of neuropeptides on the nasal cavity volume in non-sensitized guinea pigs

In non-sensitized guinea pigs, SP at 0.04, 0.4 and 4 nmol/body decreased the nasal cavity volume 10 min after the intranasal application by $14.8 \pm 1.9\%$ (means $\pm$ S.E.M., *n* = 10), $19.0 \pm 3.2\%$ (*n* = 10) and $26.4 \pm 2.8\%$ (*n* = 8), respectively. Only the decrease by SP at 4 nmol/body was significantly greater than that in the vehicle group. SP at 4 nmol/body decreased the nasal cavity volume 10 and 30 min after the intranasal application, and LY303870 (1 mg/kg) given intravenously at 30 min before the application significantly inhibited the responses (Fig. 1).

NKA at 0.04, 0.4 and 4 nmol/body decreased the nasal cavity volume 10 min after the intranasal application by $12.5 \pm 3.0\%$ (*n* = 9), $16.0 \pm 2.3\%$ (*n* = 9) and $27.6 \pm 3.5\%$ (*n* = 6), respectively. Only the decrease by NKA at 4 nmol

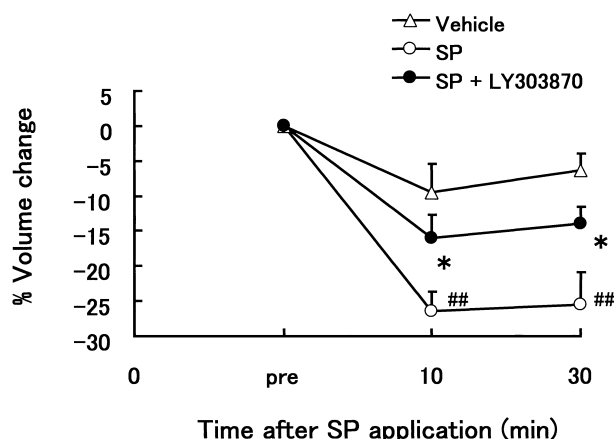


Fig. 1. Effects of LY303870 on the decreases in the nasal cavity volume after the intranasal application of substance P (SP) at 4 nmol in non-sensitized guinea pigs. Results are means $\pm$ S.E.M. of 8 animals. LY303870 (1 mg/kg) or its vehicle, saline, was administered intravenously at 30 min before the SP application. Bovine serum albumin (BSA) solution (0.1 w/v% in saline), the vehicle for the SP solution, was applied intranasally in the vehicle group. ##*P* < 0.01, as compared with the vehicle group and **P* < 0.05 as compared with the SP group.

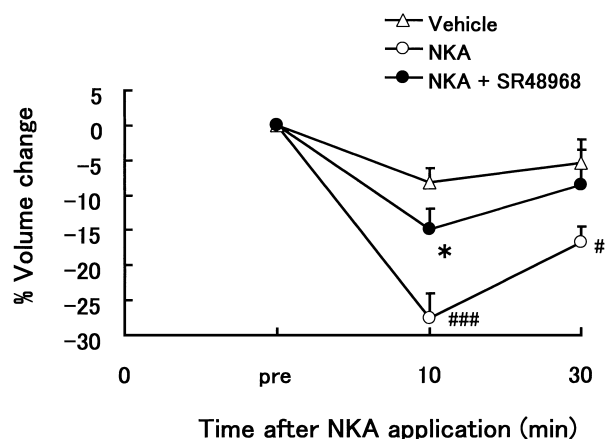


Fig. 2. Effects of SR48968 on the decreases in the nasal cavity volume after the intranasal application of neurokinin A (NKA) at 4 nmol in non-sensitized guinea pigs. Results are means $\pm$ S.E.M. of 6 animals. SR48968 (1 mg/kg) or its vehicle, 0.5 vol% Tween 80 in saline, was administered intravenously at 30 min before the NKA application. BSA solution (0.1 w/v% in saline), the vehicle for the NKA solution, was applied intranasally in the vehicle group. #*P* < 0.05 and ###*P* < 0.001, as compared with the vehicle group. **P* < 0.05, as compared with the NKA group.

/body was significantly greater than that in the vehicle group. NKA at 4 nmol/body caused the decreases in the nasal cavity volume 10 and 30 min after the intranasal application, and the intravenous administration of SR48968 (1 mg/kg) at 30 min before the application significantly inhibited the response caused 10 min after the application (Fig. 2).

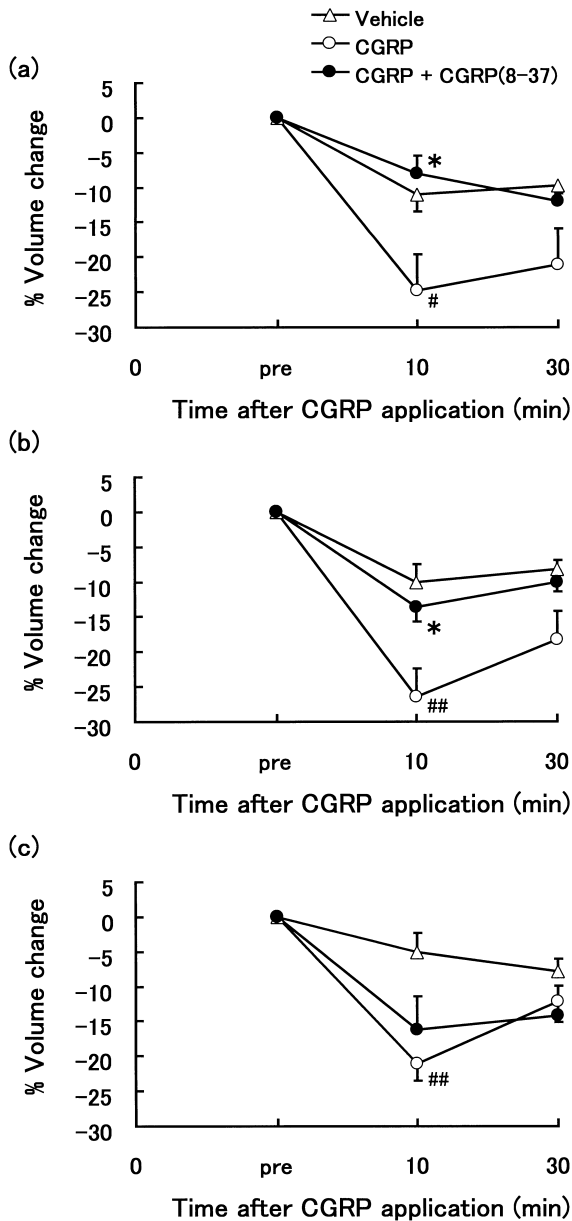


Fig. 3. Effects of calcitonin gene-related peptide (CGRP)(8–37) on the decreases in the nasal cavity volume after the intranasal application of CGRP at 4 nmol in non-sensitized guinea pigs. Results are means $\pm$ S.E.M. of 6 animals. CGRP(8–37) (50 nmol/kg) or its vehicle saline was administered intravenously at 2 min (a), 10 min (b) or 30 min (c) before the CGRP application. BSA solution (0.1 w/v% in saline), the vehicle for the CGRP solution, was applied intranasally in the vehicle group. ^{##} $P < 0.05$ and ^{###} $P < 0.01$, as compared with the vehicle group. ^{*} $P < 0.05$, as compared with the CGRP group.

CGRP at 4 nmol/body decreased the nasal cavity volume 10 min after the intranasal application, and CGRP(8–37) (50 nmol/kg) given intravenously at 2 and 10 min, but not at 30 min, before the application with CGRP significantly inhibited the response (Fig. 3).

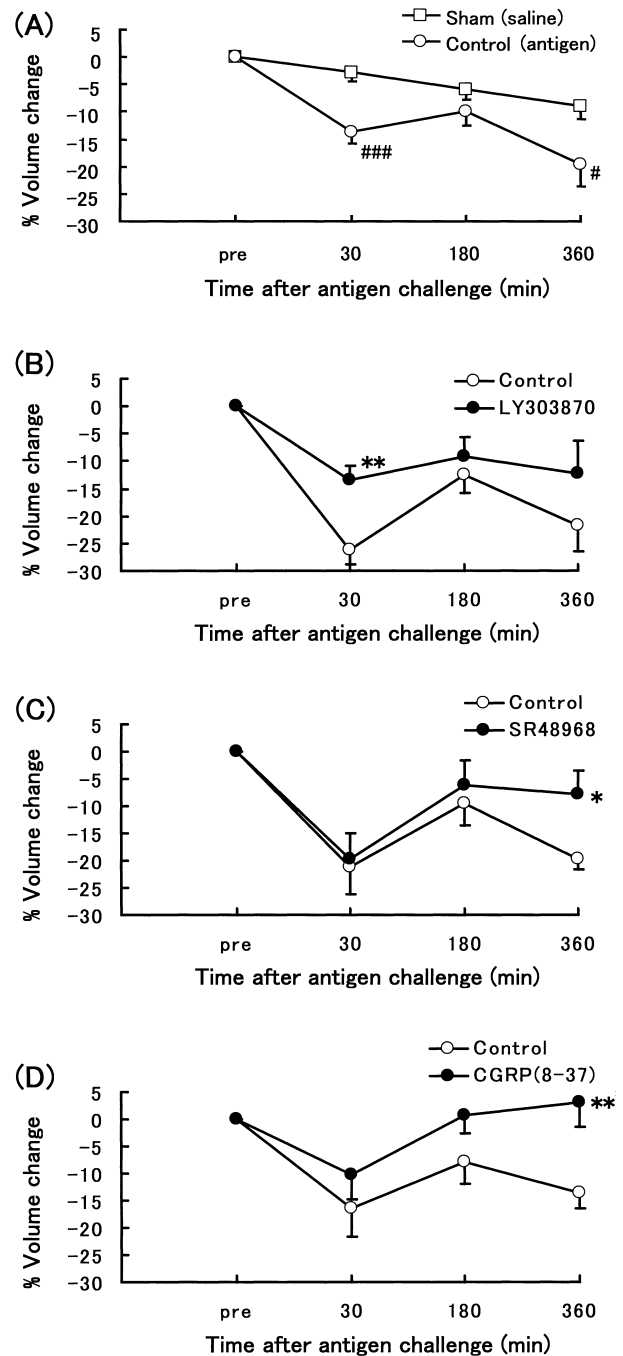


Fig. 4. The change in the nasal cavity volume after the intranasal antigen challenge (A), and the effects of LY303870 (B), SR48968 (C) and CGRP(8–37) (D) on the changes after the antigen challenge in actively sensitized guinea pigs. Results are means $\pm$ S.E.M. of 7–12 animals. A: The animals were intranasally challenged with the antigen (control) or saline (sham). B: LY-303870 (1 mg/kg) or its vehicle, saline, was administered intravenously at 30 min before the antigen challenge. C: SR-48968 (1 mg/kg) or its vehicle, 0.5 vol% Tween 80 in saline, was administered intravenously at 30 min before the antigen challenge. D: CGRP(8–37) (50 nmol/kg) or its vehicle saline was administered intravenously at 2 min before the antigen challenge. [#] $P < 0.05$ and ^{###} $P < 0.001$, as compared with the sham group (A). ^{*} $P < 0.05$ and ^{**} $P < 0.01$, as compared with the control group (B–D).

Effects of antagonists against neuropeptides on the nasal obstruction in sensitized guinea pigs

In the guinea pigs sensitized with the antigen, the percentage changes in nasal cavity volume at 30 min and 6 h after the intranasal antigen challenge were significantly greater than those after the intranasal instillation with saline (Fig. 4A). The decreases in the nasal cavity volume at 30 min and 6 h after the antigen challenge are regarded as the early phase response (EPR) and the late phase response (LPR), respectively, of the allergic nasal obstruction. LY303870 (1 mg/kg) and SR48968 (1 mg/kg), given intravenously at 30 min before the antigen challenge, significantly inhibited the allergic nasal obstruction. LY303870 inhibited the EPR, while it partly suppressed the LPR (Fig. 4B). SR48968 inhibited the LPR without affecting the EPR (Fig. 4C). Intravenous administration of CGRP(8–37) (50 nmol/kg) at 2 min before the antigen challenge significantly inhibited the LPR, while it slightly ameliorated the EPR (Fig. 4D).

DISCUSSION

The present study demonstrated that SP, NKA and CGRP caused the nasal obstruction in non-sensitized guinea pigs. The present observations in guinea pigs are consistent with the results in humans that SP, NKA and CGRP cause the nasal obstruction after the intranasal administration (7–9). We also elucidated that the antagonists against NK₁, NK₂ and CGRP1-receptors inhibited the nasal obstruction induced by SP, NKA and CGRP, respectively. These results suggest that NK₁, NK₂ and CGRP1-receptors exist in guinea pig nasal mucosa, as well as in human nasal mucosa, and that these three receptors are involved in the nasal obstruction induced by SP, NKA and CGRP, respectively. Moreover, the present study showed that the antagonists against NK₁, NK₂ and CGRP1-receptors blocked the nasal obstruction induced by the antigen-antibody reaction in guinea pigs, suggesting an involvement of neuropeptides in the allergic nasal obstruction.

We demonstrated that the intranasal antigen challenge induced the EPR and the LPR of the allergic nasal obstruction in actively sensitized guinea pigs. This observation is consistent with the result in humans showing that the nasal obstruction develops not only at 10–60 min but at 5–10 h after the intranasal antigen challenge (19).

In this study, the NK₁-receptor antagonist LY303870 significantly inhibited the EPR, while the NK₂-receptor antagonist SR48968 did not affect the EPR. The CGRP1-receptor antagonist CGRP(8–37) slightly inhibited the EPR. These results suggest that SP and CGRP are released from the nasal mucosa during the EPR following the antigen challenge. In fact, it was reported that the SP and CGRP levels in nasal lavage fluid increased immediately after

the intranasal antigen challenge in allergic rhinitis patients (20). Moreover, the tissue content of SP in guinea pig nasal mucosa correlated with the frequency of sneeze responses after the antigen-antibody reaction (21). On the other hand, the intranasal application of histamine is reported to release SP from sensory nerves in the nasal mucosa of guinea pigs (22). Yamasaki et al. (23) reported that the concentration of histamine in nasal lavage fluid increased at 10 min after the intranasal antigen challenge in sensitized guinea pigs and that the source of histamine was assumed to be mast cells in the nasal mucosa. Taken together, the EPR may be mediated, at least partly, by SP and CGRP released from the nasal sensory nerves, which is possibly stimulated by histamine, derived from mast cells, following the antigen-antibody reaction in the nasal mucosa.

The present study demonstrated that SR48968 and CGRP(8–37) significantly inhibited the LPR and LY303870 partly inhibited the LPR, suggesting that NKA, CGRP and SP are involved in the LPR. P-LTs are suggested to stimulate airway sensory nerves to release NKA, since SR48968 inhibited the bronchoconstriction induced by the LTD₄ inhalation in guinea pigs (24). P-LTs level is reported to increase in the LPR of allergic nasal obstruction in guinea pigs (25), and the source of p-LTs released during the LPR was assumed to be eosinophils (19), which accumulated into the nasal mucosa after the antigen-antibody reaction. On the other hand, in humans, histamine is reported to be released from the nasal basophils, which migrated into the nasal mucosa after the antigen challenge, in the LPR (26). Taken together, it seems that NKA, CGRP and SP are released from sensory nerves, which is possibly stimulated by p-LTs and histamine derived from migrated eosinophils and basophils, respectively, during the LPR in the guinea pig nasal mucosa.

In this study, SR48968 and LY303870 inhibited the LPR at 6 h, even following their administrations before the antigen challenge. The *in vivo* studies in guinea pigs demonstrated that SR48968 inhibited the bronchoconstriction up to 4 h after its administration (18) and that LY303870 suppressed the dural vascular hyperpermeability up to 8 h following its dosage (27), suggesting that both SR48968 and LY303870 have long duration of action in guinea pigs. It is thus reasonable to assume that SR48968 and LY303870 given before the antigen challenge inhibited the actions of NKA and SP, respectively, released in the LPR.

CGRP(8–37) also inhibited the LPR by its administration before the antigen challenge. The duration of the antagonistic action of CGRP(8–37) is considered to be less than 30 min after the intravenous administration in guinea pigs, since in the present study, CGRP(8–37) (50 nmol/kg) given at 2 and 10 min, but not at 30 min, before the intranasal CGRP challenge inhibited the nasal obstruction. It is, therefore, unlikely that CGRP(8–37) given before the antigen

challenge inhibited the action of CGRP released during the LPR. CGRP is reported to cause eosinophilia in the lung following the nebulated exposure in rats (28). Taken together, CGRP released in the EPR may lead to the aggravation of the allergic response, possibly by attracting eosinophils into the nasal mucosa. Accordingly, in the present study, the inhibition of CGRP during the EPR may have resulted in the amelioration of the LPR.

Nasal obstruction is caused by the nasal mucosal swelling, which is induced either by mucosal edema, resulting from increased vascular permeability, or by dilation of capacitance vessels, leading to engorgement of blood in the nasal mucosa (23). The presence of intraluminal secretions also contributes to nasal obstruction (29). Several studies have elucidated that SP causes the nasal vascular hyperpermeability, the dilation of nasal blood vessels and glandular secretion, while NKA mainly induces increased vascular permeability and CGRP predominantly exhibits arteriolar vasodilatory properties (5, 7, 8). These results suggest that, in the present study, exogenously applied SP, NKA and CGRP caused the nasal obstruction by acting on either nasal blood vessels or nasal glands. Moreover, endogenously released SP, NKA and CGRP from sensory nerves, possibly by the action of histamine or p-LTs, are suggested to have contributed to the nasal obstruction following the antigen-antibody reaction in the nasal mucosa.

In conclusion, we elucidated that SP, NKA and CGRP induced the nasal obstruction in guinea pigs, and the responses induced by three neuropeptides were blocked by the NK₁, NK₂ and CGRP1-receptor antagonists, respectively. Furthermore, we demonstrated that the antagonists against NK₁, NK₂ and CGRP1-receptors inhibited the nasal obstruction induced by the antigen-antibody reaction in guinea pigs. These results suggest that neuropeptides are involved in allergic nasal obstruction.

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